Supporting Information

Horiuchi et al. 10.1073/pnas.1111317109

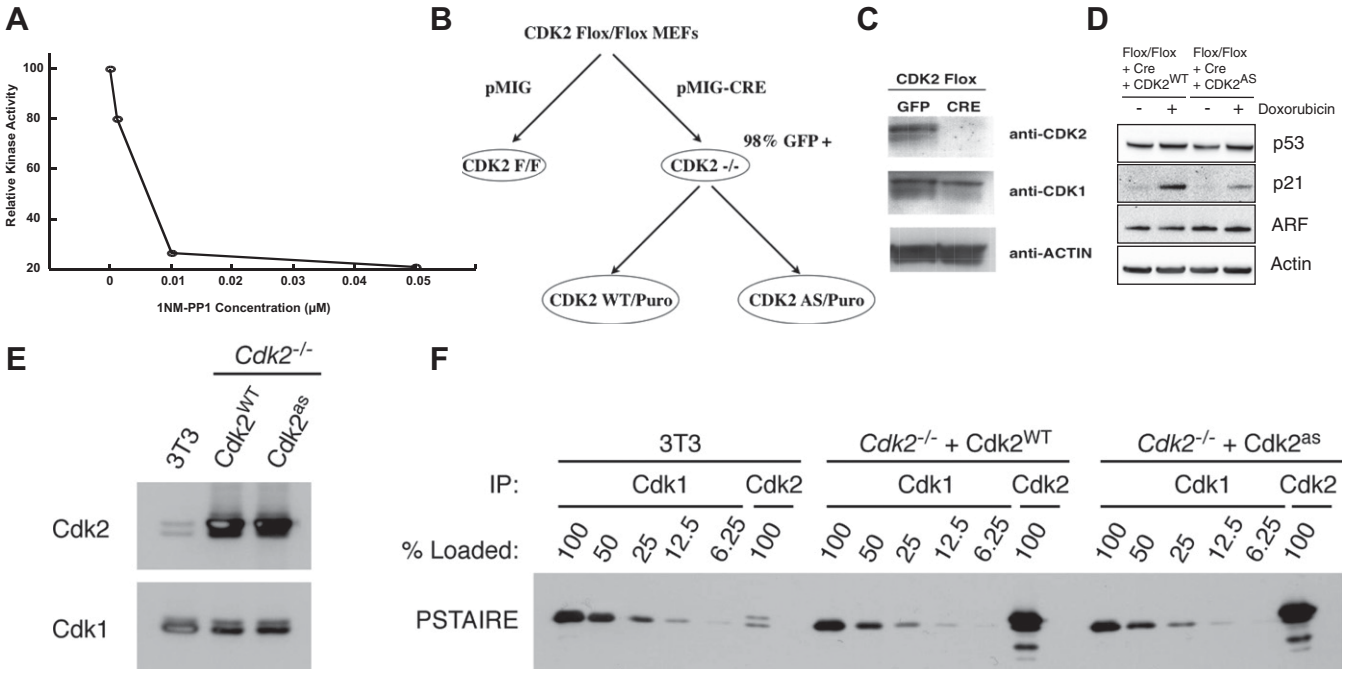

Fig. S1. Endogenous CDK2 is deleted in CDK2$^{flox/flox}$ MEFs and replaced with CDK2^{AS} or CDK2^{WT} alleles. (*A*) Quantification of CDK2-AS kinase inhibition from Fig. 1*D*. (*B*) Schematic of the generation of MEFs expressing the WT or AS allele of CDK2. Puro, Puromycin. (*C*) Cre-recombinase was retrovirally expressed in Cdk2$^{flox/flox}$ MEFs, and depletion of Cdk2 protein expression is shown by Western blotting. (*D*) Western blot analysis of the expression of p53, p21, and ARF in the MEFs with CDK2^{WT} or CDK2^{AS} in the presence or absence of doxorubicin. (*E*) Amount of Cdk1 and Cdk2 expressed in asynchronously growing mouse 3T3 fibroblasts and Cdk2$^{-/-}$ MEFs expressing Cdk2^{WT} or Cdk2^{AS} was determined by immunoblotting. (*F*) To determine the amount of Cdk2 expressed compared with Cdk1, extracts from 3T3 cells and Cdk2$^{-/-}$ MEFs expressing Cdk2^{WT} or Cdk2^{AS} were denatured, and Cdk1 and Cdk2 were subsequently immunoprecipitated. The relative amount of each CDK in the extracts was determined by immunoblotting using a PSTAIRE antibody, which detects both kinases equally well (1). IP, immunoprecipitation.

1. Dobashi Y, et al. (1998) Active cyclin A-CDK2 complex, a possible critical factor for cell proliferation in human primary lung carcinomas. *Am J Pathol* 153:963–972.

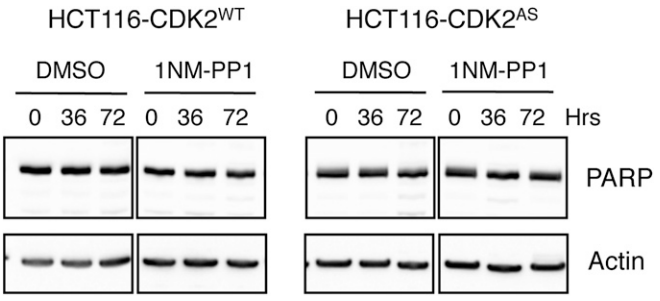

Fig. S2. Western blot analysis of PARP cleavage in HCT116 cells. The HCT116 cells carrying either the CDK2^{WT} allele or CDK2^{AS} allele were treated with 1NM-PP1 for the indicated amount of time and assessed for the cleavage of PARP.

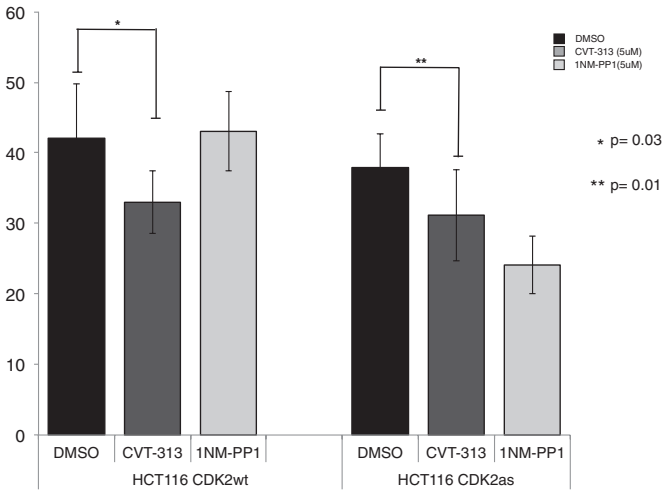

Fig. S3. HCT116-CDK2^{WT} and HCT116-CDK2^{AS} cells respond similarly to a commercially available CDK2 inhibitor. HCT116 cells carrying either the CDK2^{WT} allele or CDK2^{AS} allele were treated with 5 μM CVT-313, and the percentage of cells in S phase was assessed using a FACS-based BrdU incorporation assay (n = 4). Error bars = SEM. *Statistical significance as determined by a paired two-tailed t test.

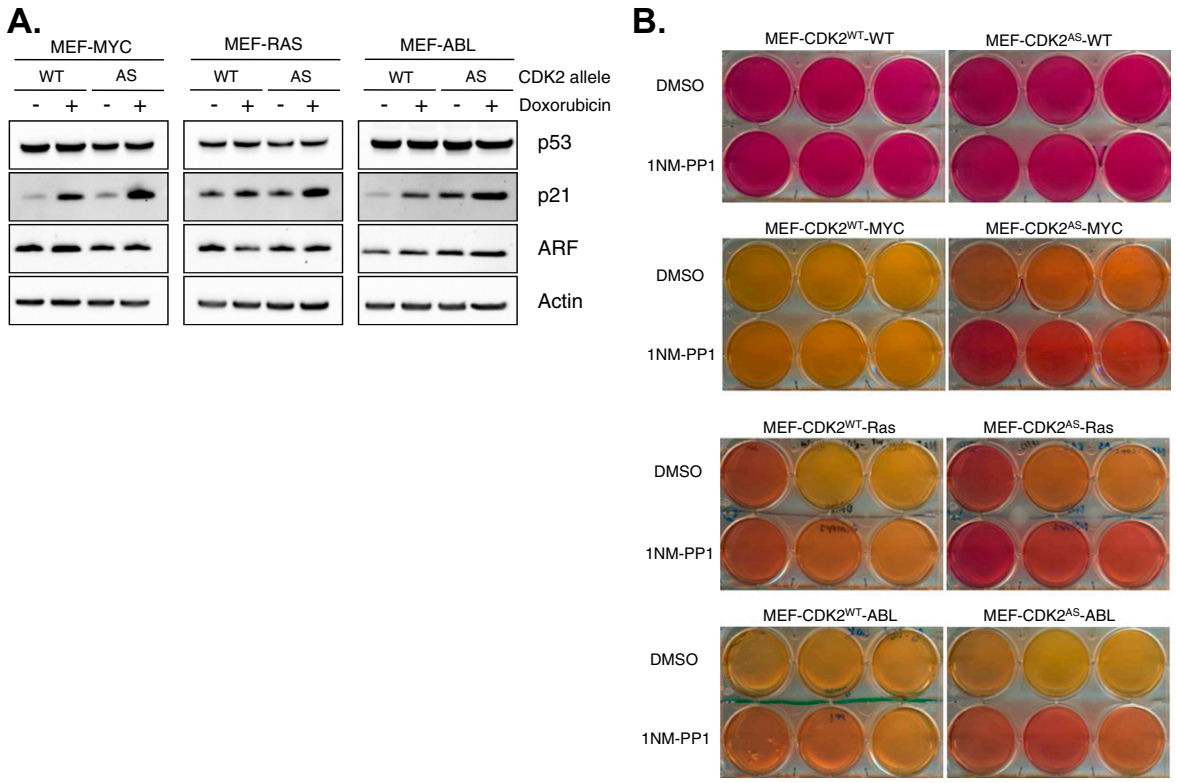

Fig. S4. Anchorage-independent growth of the oncogene-transformed MEFs. (*A*) Western blot analysis of the expression of p53, p21, and ARF in the oncogene-transformed MEFs. (*B*) Anchorage-independent growth in soft agar. The oncogene-transformed MEFs were pretreated with DMSO or 5 μM 1NM-PP1 for 72 h. Following pretreatment, the cells were plated in 0.32% soft agar containing DMSO or 5 μM 1NM-PP1 and grown for 3–5 wk depending on the oncogene used.

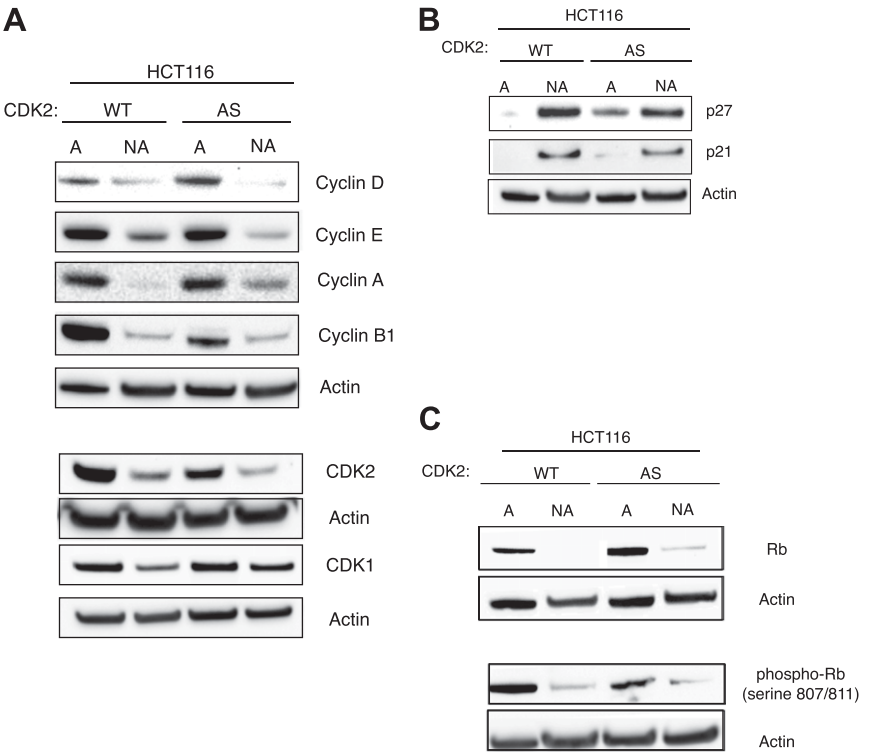

Fig. S5. Altered expression of cell cycle regulator proteins in HCT116 cells grown in adherent (A) vs. nonadherent (NA) conditions. (*A*) Western blot analysis of the expression of cyclins, CDK2, and CDK1 in the HCT116-CDK2^{WT} or HCT116-CDK2^{AS} cells grown in adherent vs. nonadherent conditions. (*B*) Western blot analysis of the expression of p27 and p21 in HCT116-CDK2^{WT} or HCT116-CDK2^{AS} cells grown in adherent vs. nonadherent conditions. (*C*) Western blot analysis of the Rb Ser-807/811 phosphorylation in the HCT116-CDK2^{WT} or HCT116-CDK2^{AS} cells grown in adherent vs. nonadherent conditions.